Pigment Epithelium-Derived Factor as a New Marker of Metabolic Syndrome in Caucasian Population

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Authors present that serum pigment epithelium derived factor (PEDF) is an independent marker of metabolic syndrome in Caucasian

population. PEDF was measured with new ELISA sandwich test. J. Clin. Lab. Anal. 24:17–19, 2010. © 2010 Wiley-Liss, Inc.

Key words: PEDF; metabolic syndrome

INTRODUCTION

Pigment epithelium derived factor (PEDF) was discovered as a 50 kDa protein released by cultured pigment epithelial cells from the fetal human retina. It is synthesized especially in the liver, but also in a wide range of human tissues such as the lung, brain, kidney, and adipose tissue. PEDF is an extracellular multifunctional protein belonging to the serpin family and has demonstrable neurotrophic, gliastatic, antiangiogenic, and antitumorigenic properties. Serpins are a group of serine protease inhibitors, some of which have also reported to exhibit neurotrophic activity. PEDF is a potent inhibitor of angiogenesis and shows differentiating activity in cells derived from retina and central nervous system (1,2), and blocks fibrogenesis in diabetic kidneys through inhibition of transforming growth factor beta and connective tissue growth factor expression and function (3). Moreover, it inhibits endothelial cell injury in vitro suggesting the involvement of PEDF in atherosclerosis (4). In cell culture and in animal models, PEDF inhibited endothelial cell growth and migration and suppressed ischemia-induced neovascularization, whereas in the liver, the expression of PEDF is associated with body muscularity and obesity. PEDF also possesses antioxidant and anti-inflammatory properties (1). Recently it was discovered that PEDF levels are associated with metabolic syndrome and several coronary risk factors in a Japanese population. In our study we examined whether PEDF serum levels were

associated with metabolic syndrome risk factors in a Caucasian population.

MATERIALS AND METHODS

Subjects

The study was designed as a cross-sectional study and was approved by the ethical commission of the Hospital Sternberk, Czech Republic. A total of 112 Caucasians (age: 66 ± 9 years, 40 males and 72 females) with a different number of risk factors for metabolic syndrome were enrolled in this study. According to the National Cholesterol Education Program Adult treatment Panel III (NCEP ATP III), the metabolic syndrome is diagnosed when three or more of the following criteria are found in a subject: (1) waist circumference equal to or greater than 88 cm in women or equal to or greater than 102 cm in men; (2) elevated triglycerides (equal to or greater than 1.7 mmol/l); (3) reduced high density cholesterol (HDL-cholesterol) (men: less than 1.0 mmol/l and women: less than 1.3 mmol/l); (4) elevated blood pressure (equal to or greater than 130/85 mm Hg or being under therapy with blood pressure lowering

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drugs); (5) Elevated fasting glucose (equal to or greater than 5.6 mmol/l). Exclusion criteria of the study were as follows: acute or chronic systemic inflammation (medical history, C-reactive protein > 10 mg/l, leukocytosis, clinical examination), diabetes mellitus treated with per oral antidiabetic drugs or insulin, liver diseases with transaminases levels above five times the normal upper limit, kidney diseases with creatinine levels above 130 μ mol/l, and/or proteinuria exceeding 0.5 g/l.

METHODS

Anthropometric, clinical (height, weight, BMI, waist circumference, systolic, and diastolic blood pressure), and laboratory analyses were performed. Fasting blood samples were drawn under aseptic conditions from vena cubiti, after several minutes' rest in half-sitting position. Serum samples were separated by centrifugation at 4°C at 3000g for 20 min and subsequently used for analysis or frozen at -80°C. Insulin, fasting glucose, cholesterol, LDL-cholesterol, HDL-cholesterol, total cholesterol, triglycerides, insulin sensitivity check index (Quicki) were measured.

Measurement of PEDF

PEDF serum levels were measured with a new commercially available ELISA assay (from Biovendor-Laboratori medicina, Modrice, Czech Republic), which does not require any sample treatment with urea. It is a sandwich ELISA kit designed for measuring the total amount of PEDF in serum and plasma samples. No pretreatment of samples with urea is required in comparison with the assay from Millipore. Briefly, 100 μ l aliquots of standard (prepared from recombinant PEDF protein) and samples (diluted 8000 times) were added to the wells precoated with specific polyclonal antibody. In the subsequent step, the biotin-labeled anti-PEDF antibody was added to each well. Following incubation with streptavidin-HRP conjugate and substrate solution, the color development was stopped by adding 0.1 M sulfuric acid and absorbance was read at 450 nm using a microplate reader. The limit of detection of the assay was 0.045 ng/ml and the inter-assay and intra-assay coefficients of variation were always less than 8%.

Statistical Analysis

The data obtained were processed by means of the software Medcalc (Medcalc, Mariakerke, Belgium). The value $P < 0.05$ was considered as statistically significant. Because of anomalous data distribution in evaluated parameters, Spearman's correlation coefficients were used to establish the association between PEDF levels

and the other parameters. Measured markers were compared with Kruskal-Wallis test (anomalous data distribution). ROC analysis for PEDF serum values' ability to predict metabolic syndrome was performed (Fig. 1). All data are presented as medians and/or mean $\pm$ standard deviation.

RESULTS

PEDF showed abnormal distribution, ranging from 4.7 mg/l to 32.5 mg/l, with a median of 13.2 mg/l. PEDF serum levels did not differ in men and women, see Table 1.

Analyses revealed that PEDF is correlated with BMI ($r = 0.32$, $P < 0.01$), CRP ($r = 0.33$, $P < 0.01$), insulin ($r = 0.082$, $P < 0.02$), and Quicki ($r = -0.22$, $P < 0.05$). Individuals with metabolic syndrome (NCEP ATP III criteria, $n = 72$) had significantly higher PEDF values (median 15.6 mg/l vs. 11.2 mg/l, large samples test statistic $Z = 2.33$, $P < 0.01$) than healthy subjects (without components of metabolic syndrome, $n = 40$).

PEDF sensitivity and specificity for the presence of metabolic syndrome were 57.9 respectively 86.7, at a cut-off value of 14.9 mg/l (AUC 0.75, 95% CI 0.55–0.87, LR+4.34).

Multivariate analyses revealed that no measured parameters were significant independent determinants of PEDF serum levels. However, BMI-adjusted PEDF levels were significantly higher in individuals with metabolic syndrome than without. We observed a significant correlation between serum PEDF and the number of metabolic syndrome criteria/components and

Fig. 1. ROC analysis of PEDF and metabolic syndrome presence.

TABLE 1. Laboratory Characteristics of the Study Group—Subjects with and without Metabolic Syndrome

Parameter	Unit	Mean	SD	Median without MetSy	Median with MetSy	RR
PEDF total	mg/l	10.8	6.4	11.2	15.6	$P < 0.01$
PEDF men	mg/l	10.9	7.1	10.7	16.4	$P < 0.01$
PEDF women	mg/l	10.3	6.3	10.2	15.9	$P < 0.01$
BMI	U	23.4	3.2	25.0	34.6	$P < 0.01$
CRP	mg/l	4.8	1.2	4.2	3.9	$P = 0.54$
Insulin	IU/l	11.3	4.4	12.3	18.1	$P < 0.01$
Glucose	mmol/l	4.2	1.7	4.6	6.8	$P = 0.04$
Age	Years	64.3	8	64	68	$P = 0.39$

RR, differences between subgroup by the metabolic syndrome presence; MetSy, metabolic syndrome.

this relationship was confirmed by frequency table (χ^2 62.1, $P < 0.01$).

DISCUSSION

The cross-sectional study, designed to elucidate the association of PEDF serum levels with the metabolic syndrome, revealed that Caucasian individuals with components of metabolic syndrome had significantly higher PEDF serum levels than healthy subjects and that PEDF was an independent marker of metabolic syndrome with sufficient diagnostic efficacy. PEDF was correlated with BMI, CRP, diastolic blood pressure, insulin, and Quicki.

The role of PEDF in the metabolic syndrome is not completely understood. Recently it was reported that plasma PEDF level in diabetic patients was significantly higher compared to that of controls, and it was markedly higher in patients with proliferative diabetic retinopathy. Diabetic retinopathy and nephropathy are microvascular complications associated with diabetes. Chronic, low-grade inflammation is responsible for diabetic microangiopathy, such as retinopathy and nephropathy. It is known that PEDF has a protective role against vascular damage by suppressing proliferative inflammatory responses to injuries in endothelial cells. PEDF levels may increase as a counteractive system against coronary risk factors in the metabolic syndrome. The elevation of PEDF levels could be regarded as an epiphenomenon of the atherosclerotic process rather than a pathophysiological factor in the development of the metabolic syndrome. The presence of PEDF transcripts in a broad range of adult human tissues, especially in liver, may account for the high serum levels of PEDF. The liver has been hypothesized to be the main origin of PEDF in circulation (1), and the expression level of PEDF in porcine liver has been shown to be associated with body muscularity and

obesity. PEDF is expressed also in adipose tissue, and reports indicate that PEDF is down-regulated during adipocyte differentiation and increased in obesity (5). Reports from several small clinical studies indicate that serum PEDF is elevated in Japanese subjects with the metabolic syndrome (1), and that PEDF is positively associated with obesity, particularly with visceral fat depot, in Caucasian subjects (6). These results are in agreement with our findings. Moreover, similar to our results, Yamagishi et al. reported that PEDF level increases with the number of components of the metabolic syndrome.

In summary, the PEDF level in blood is elevated in patients with metabolic syndrome and might be useful as a laboratory parameter for its diagnosis.

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